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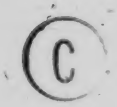
ALLOZYME ANALYSIS OF LODGEPOLE PINE ALONG ELEVATIONAL TRANSECTS IN ALBERTA

by

Gregory John Lee



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ABSTRACT

Genetic variability and population structure of lodgepole pine (*Pinus contorta* Dougl. ssp. *latifolia* Engelm.) along elevational transects in Alberta was analyzed electrophoretically for 20 enzymes coded by 29 structural loci. Three populations were sampled along each of three elevational transects along the eastern slopes of the Rocky Mountains.

Seventy-six alleles were scored at the 29 loci. Only one locus, LAP, was monomorphic in all nine populations. Mean expected heterozygosity ranged from 0.130 to 0.193 over the nine populations. In all cases, expected heterozygosity exceeded the observed number of heterozygotes. Analysis of F-statistics showed a 19.9% deficiency of heterozygotes over the nine populations.

The percentage of polymorphic loci ranged from 58.6 to 86.2. The mean number of polymorphic loci decreased from north to south, while the high elevation populations were the least polymorphic. A heterogeneity chi-square analysis indicated that there was significant heterogeneity among all nine populations, among the three transects, and among the elevations sampled. Most (95.3%) of the detected genetic variability resided within populations.

Mean genetic distance among populations was 0.008. Phenetic clustering and discriminant analysis showed that the low elevation population of the north transect may be significantly different from the other eight populations.

Little among-population differentiation has occurred, but there is evidence of family structure within populations as illustrated by high levels of inbreeding.

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Table of Contents

Chapter		Page
I.	INTRODUCTION	1
II.	MATERIALS AND METHODS	6
	A. FIELD COLLECTIONS	6
	SAMPLE LOCATIONS	6
	CONE COLLECTIONS	6
	B. LAB ANALYSIS	10
	C. DATA ANALYSIS	13
III.	RESULTS	14
IV.	DISCUSSION	30
V.	LITERATURE CITED	35
VI.	APPENDIX I.	46
	A. STAND DESCRIPTIONS	46
VII.	APPENDIX II.	47
	A. BUFFERS USED IN STAINING SOLUTIONS	47
	B. ENZYME STAINING RECIPES	49
	C. GEL AND ELECTRODE BUFFERS	57

LIST OF TABLES

Table		Page
1	Location of lodgepole pine populations in Alberta.	8
2	Enzymes assayed and procedures used.	12
3	Allelic variation at 29 gene loci in nine populations of lodgepole pine in Alberta.	15-19
4	Summary of heterogeneity chi-square analysis for 28 gene loci of lodgepole pine in Alberta.	20
5	Summary of heterogeneity chi-square analysis for 28 gene loci of lodgepole pine in Alberta.	21
6	Common allele frequency of selected loci of lodgepole pine averaged over three latitudinal transects in Alberta.	22
7	Common allele frequency of selected loci of lodgepole pine averaged over three elevations in Alberta.	22
8	Genetic variability measures of nine lodgepole pine populations in Alberta.	24
9	Estimates of F-statistics at 28 loci of nine lodgepole pine populations in Alberta.	25
10	Genetic distance (below the diagonal; from Nei 1978) and geographic distance (km; above the diagonal) for pairwise comparisons among nine populations in Alberta.	26
11	F-statistics from comparable studies on coniferous trees.	33
I	Stand summary data for nine lodgepole pine populations in Alberta.	46

LIST OF FIGURES

Figure		Page
1	Locations of lodgepole pine transects in Alberta.	7
2	Sampling scheme used for lodgepole pine elevational transect study.	9
3	UPGMA-derived phenogram based on Nei's unbiased genetic distance measure.	28
4	Population centroids on the first two discriminant axes.	29

I. INTRODUCTION

Forest tree populations, like virtually all plant populations, do not fulfill the assumptions of the Hardy-Weinberg model. Forest tree populations are not infinitely large and their sizes are rarely constant, so fluctuations in allele frequencies can occur by chance. Random mating, or panmixia, is less likely to occur in plant populations than in some species with highly mobile individuals. Forest tree populations also are subject to the systematic evolutionary forces of migration, mutation, and natural selection, all of which can cause non-random or directional changes in allele frequencies.

The results of these events can lead to disruptions of random gamete combinations, which tend to divide a population into genetic subpopulations, reduce the frequency of heterozygous genotypes and facilitate local differentiation (Schaal 1975). This subdivision of a population is referred to as population structure. Changes in allele frequencies among subpopulations are manifestations of population structure.

Wright (1943, 1946) developed the term neighborhood, which represented the area from which any two parents could have come with equal probability. Here, individuals in the population are neighbors in the sense that their gametes may come together; in time, this may lead to numerous small neighborhoods in the population. Many authors have since

used the term neighborhood when talking of plant population structure (e.g., Endler 1977, 1979, Levin and Kerster 1969, and Schaal 1980). The neighborhood concept also has been used to describe genetic structure of forest tree populations (Bannister 1958, Coles and Fowler 1976, Guries and Ledig 1981, Ledig 1974, Sakai and Miyazaki 1972, Sorensen 1973, Tigerstedt 1974). These neighborhoods, or genetic subpopulations, can also be formed as a result of factors such as physical isolation, breeding system, environmental heterogeneity, and population demography (Guries and Ledig 1981).

Harris (1966) and Lewontin and Hubby (1966) first used electrophoresis as a tool to study genetic variation of populations. Electrophoresis can be used directly to assess genetic variation. Electrophoretic techniques offer a number of advantages over other biochemical or quantitative methods. These include: a) genetic inheritance of electrophoretically detectable traits can be easily demonstrated, b) most enzyme loci are codominant and therefore gene frequencies can be calculated without the need to make genetic crosses, and c) estimates of genetic variation can be compared directly among populations (Hamrick *et al.* 1979).

In recent years, a vast number of electrophoretic studies of genetic variation in forest tree populations have been performed (e.g. Dancik and Yeh 1983, Fowler and Morris 1977, Mitton *et al.* 1977, Wheeler and Guries 1982, Yeh and

Layton 1979, and Yeh and O'Malley 1980). Coniferous trees are particularly useful for electrophoretic analysis, because the haploid megagametophyte tissue in the seeds has the same genetic makeup as the female gamete. Thus, the Mendelian genetics of allozyme (allelic isozymes) variants can be confirmed without making controlled crosses, because allozymes are expected to segregate in 1:1 ratios in the megagametophytes of heterozygous mother trees (Adams and Joly 1980).

Electrophoresis, then, can be an effective tool for studying population structure of forest trees. Several methods for measuring population structure with electrophoretic data have been devised. These include F-statistics (Nei 1977, Wright 1949, 1951, 1965), chi-square tests for heterogeneity (Workman and Niswander 1970), and genetic distance (Cavalli-Sforza and Edwards 1967, Nei 1972, Rogers 1972). Forest trees are reported to have higher levels of variation than other plants (Hamrick 1979). However, most of this variation resides within populations rather than among populations (Dancik and Yeh 1983, Guries and Ledig 1982, Hiebert and Hamrick 1983, O'Malley *et al.* 1979, Wheeler and Guries 1982, Yeh and Layton 1979, Yeh and El-Kassaby 1980, Yeh and O'Malley 1980). While there may be some genetic structure in forest tree populations, several authors have reported that there is less structure in these populations than in those of other plants (Guries and Ledig 1981, 1982, Hamrick *et al.* 1981, Yeh and Layton 1979, Yeh

and O'Malley 1980). Forest trees typically have large population sizes, a predominantly outcrossing mating system, and the potential for long distance gene flow via wind pollination, all of which may contribute to low population subdivision (Wheeler and Guries 1982).

In this study, I investigated genetic variation and the population structure of lodgepole pine (*Pinus contorta* Dougl. ssp. *latifolia* Engelm.) along elevational transects along the eastern slopes of the Rocky Mountains in Alberta. Lodgepole pine is one of the most widespread of North American conifers. Critchfield (1957) conducted an extensive survey of morphological traits of *Pinus contorta* and suggested that there were four subspecies. These are the coastal group (*Pinus contorta* ssp. *contorta*), inland or Rocky Mountain group (*Pinus contorta* ssp. *latifolia*), Mendocino white plains group (*Pinus contorta* ssp. *bolanderi*), and the Sierra Nevada group (*Pinus contorta* ssp. *murrayana*). Only the subspecies *latifolia* was studied here and is referred to as lodgepole pine (*Pinus contorta* ssp. *latifolia*).

Lodgepole pine has an extensive and diverse natural distribution. It is found from the Yukon south to Colorado, spanning some 26 degrees of latitude and 3900 meters of elevation (Critchfield 1980). In Alberta, lodgepole pine can be found from 800 meters in elevation to timberline (Horton 1956). It generally grows in mountainous regions, but may be found under a variety of topographic conditions (Fowells

1965).

The specific objectives of this study were:

(i) to summarize the amount and organization of genetic variation in lodgepole pine in the foothills of Alberta,

(ii) to analyze the population structure of lodgepole pine within and among populations,

(iii) to determine whether there was any pattern of genetic variation associated with obvious synthetic environmental or spatial differences such as latitude or elevation.

II. MATERIALS AND METHODS

A. FIELD COLLECTIONS

SAMPLE LOCATIONS

Field work was conducted in the summer of 1983. Three elevational transects were established along the eastern slopes of the Rocky Mountains (Figure 1). From north to south the transects are referred to as Robb, Nordegg, and Cochrane, respectively. At each transect, three populations were selected at low, intermediate, and high elevation to represent the range of elevation encompassed by the species (Table 1).

CONE COLLECTIONS

Cones were collected at all nine populations following the same sampling strategy. A 70 m x 70 m grid was set up consisting of seven (10 m x 10 m) subplots (Figure 2). Close to the center of each subplot, a dominant or co-dominant tree was felled and the cones were collected from it. This gave a total of 49 trees per population and a grand total of 441 trees from all nine plots. In addition, at Robb Mid, Nordegg Low, and Cochrane Low all the trees within the center plot of the grid (subplot number 25) were felled and cones were collected. Also, diameter at breast height, total tree height, age, and stand density around the selected tree were collected (See Appendix I for stand descriptions).

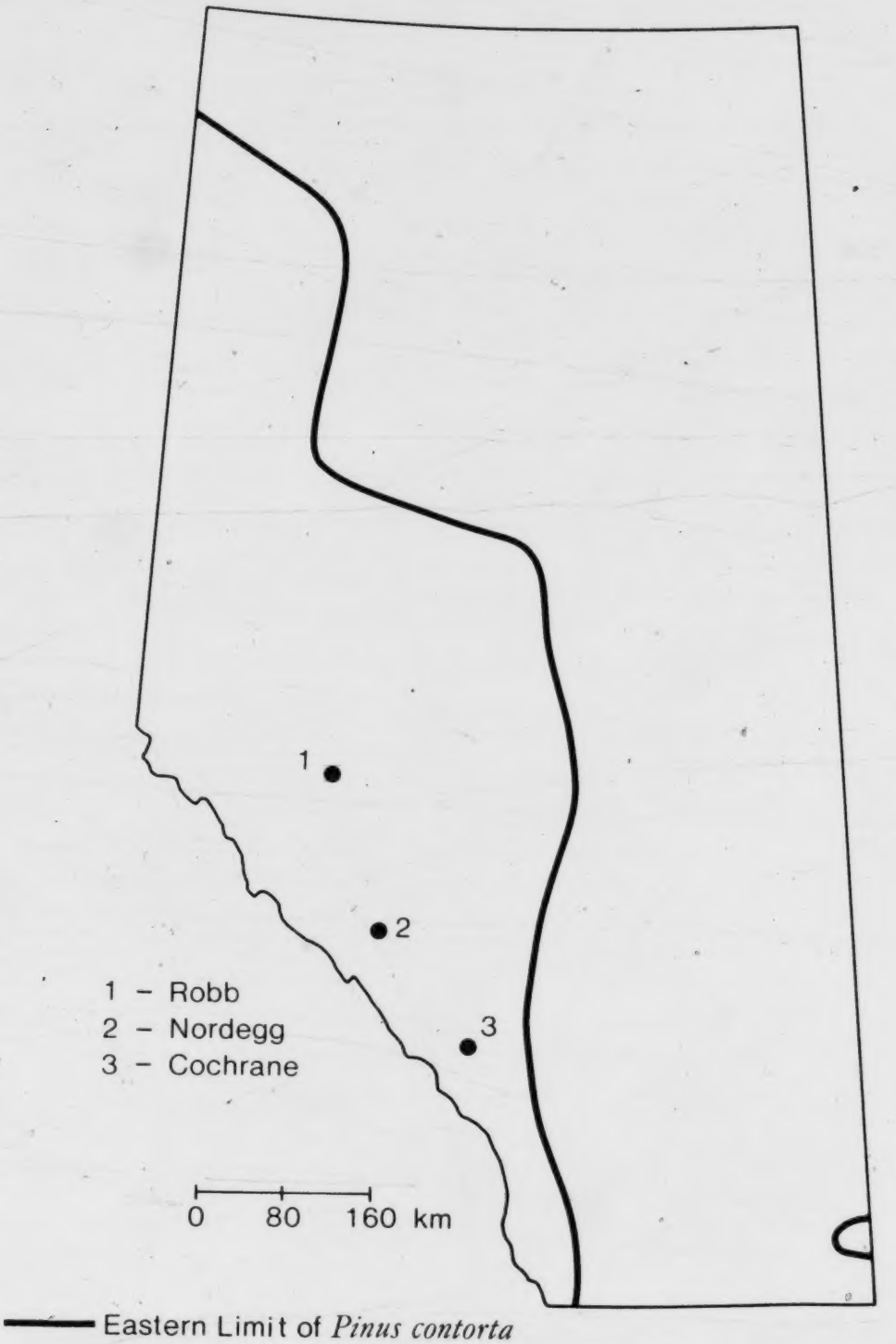


Figure 1. Locations of the lodgepole pine elevational transects in Alberta

Table 1. Location of lodgepole pine populations in Alberta.

POPULATION	LAT.	LONG.	ELEV. m.	NO. TREES
1. ROBB LOW	53°25'	116°40'	1050	49
2. ROBB MID	53°10'	117°15'	1300	49
3. ROBB HIGH	53°17'	117°20'	1660	49
4. NORDEGG LOW	52°30'	116°04'	1100	49
5. NORDEGG MID	52°26'	116°15'	1520	49
6. NORDEGG HIGH	52°20'	116°15'	1700	49
7. COCHRANE LOW	51°40'	115°12'	1300	49
8. COCHRANE MID	51°46'	115°20'	1650	49
9. COCHRANE HIGH	51°50'	115°15'	1910	49

Figure 2. Sampling scheme used for lodgepole pine elevational transect study.

70m

10m

1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31	32	33	34	35
36	37	38	39	40	41	42
43	44	45	46	47	48	49

10m

70m

Sample size was calculated assuming that the gene frequencies of two alleles were equal, thus reducing the variance. Previous electrophoretic studies have used sample sizes of approximately 30 to detect any rare alleles. For this study, a conservative minimum population size was calculated as 48 using the formula: $N = (Z/E)^2 pq$, where Z is the distribution table value, E is the error value, and p and q are the gene frequencies assumed to be 0.5 each. Therefore, the 7 x 7 grid gave a sample population size of 49 and was useful in analyzing genetic sub-structuring in the sample population.

B. LAB ANALYSIS

Seeds were extracted from the serotinous cones using the method described by Cheliak (1983). Cones were placed in an oven at 180° C for two minutes to break the resin bonds of the cone scales. The cones then were placed in an oven at 60° C for approximately 8 hours to reflex the scales more fully. The seeds were then extracted by lightly tapping each cone on a hard surface.

Seeds were allowed to imbibe distilled water in petri dishes at room temperature for approximately 24 hours. They were then either assayed immediately or kept refrigerated for up to three days before assaying. Seed coats were removed, embryos were separated and discarded, and individual megagametophytes were ground in auto-analyzing cups (0.5 ml) with 40μl of extraction buffer (Yeh and

O'Malley 1980). The homogenate was then absorbed onto 13 x 1 mm filter paper wicks. With eight megagametophyte tissue samples used from each parent tree, the probability of misclassifying any heterozygote was very small (0.0078).

The wicks with the homogenate were then inserted into a slice in a starch gel 3 cm from the cathodal end. Starch gels (12.5%) were prepared from Connaught Hydrolyzed Starch (Willowdale, Ontario). Gel and electrode buffer recipes are listed in Appendix II. Four different buffer systems were used to resolve the 20 enzyme systems and the 29 structural loci studied (Table 2).

Electrophoresis was performed under refrigeration at approximately 4° C. The gels were run at half voltage for 15 minutes, at which time the wicks were removed, crushed ice packs were applied to the upper surface of the gel, and total running voltage was applied.

Electrophoresis was performed until the red food coloring marker dye (buffer systems A and C) or the borate front (buffer systems B and D) had migrated approximately 6-8 cm. Following electrophoresis, the gels were sliced into 1 mm thick horizontal slices. Each gel slice was stained for one particular enzyme; the gels were either incubated at 37° C in the dark or left at room temperature to develop. Enzyme staining recipes are indicated in Appendix II.

Gene loci were inferred from patterns of segregation observed from the enzyme systems studied. The most common allele at each locus was designated as number 1. Different

Table 2. Enzymes assayed and procedures used.

ENZYME	ABBREVIATION	BUFFER SYSTEM*	NO. OF LOCI SCORED
Aspartate aminotransferase	AAT	D	3
Aconitase	ACO	A	1
Alcohol dehydrogenase	ADH	B	1
Adenylate kinase	AK	A	1
Aldolase	ALD	B	1
Acid phosphatase	APH	C	3
Colorimetric esterase	CE	B	1
Diaphorase	DIA	A	1
Fluorescent esterase	FLE	A	1
Glutamate dehydrogenase	GDH	B	1
Glucose 6-phosphate dehydrogenase	G6P	C	1
Hexoseaminidase	HA	A	1
Hexokinase	HK	A	1
Isocitrate dehydrogenasae	IDH	A	1
Leucine aminopeptidase	LAP	B	1
Malate dehydrogenase	MDH	A	4
Phosphoglucomutase	PGM	A	1
Phosphoglucose isomerase	PGI	C	2
6-Phosphogluconic dehydrogenase	6PG	C	2
Sorbitol dehydrogenase	SDH	B	1

* Buffer systems:

A - Tris/citrate pH 7.0 (Siciliano and Shaw, 1976)
B - Tris/citrate: Li/borate pH 8.1 (Ridgway, 1970)
C - L-histidine/tris (SPB) pH 7.0 (Florence, 1981)
D - Poulik pH 8.1 (Schaal and Anderson, 1974)

alleles at a locus were confirmed by measurement relative to a corn (Spancross hybrid) standard. Each enzyme was designated by an abbreviation (Table 2), a number indicating the locus, and another number which indicated the allele at that locus. For example, MDH-3 represents the third malate dehydrogenase locus.

C. DATA ANALYSIS

The computer program BIOSYS1 was used to analyze the allozyme data (Swofford and Selander 1981). For this study, it was used to compute allele frequencies and genetic variability measures, to calculate F-statistics, to perform heterogeneity chi-square analysis, to calculate several similarity and distance coefficients, and to construct phenograms using cluster analysis.

Two multivariate methods were used to analyze the data set. Cluster analysis was performed to partition the sample populations into separate or overlapping homogeneous classes. Discriminant analysis was used to find the linear combination of variables that best separated or discriminated among pre-defined groups. The most common allele method of coding the data set was used as described by Smouse and Neel (1977). All significance tests were made at the 0.05 level.

III. RESULTS

Of the 29 loci assayed, 28 were polymorphic in at least one of the nine populations studied, and many were highly polymorphic, with up to five alleles (Table 3). Significant allele frequency differences were observed among populations at 14 loci (Table 4), and among the center plots of Robb Mid, Nordegg Low, and Cochrane Low, and all trees of the three populations (Table 5). One allele was generally predominant at the remaining loci. Significant heterogeneity of mean allele frequencies was observed among transects and elevations (Table 4). At some loci, allele frequencies increased or decreased with latitude or elevation (Tables 6 and 7). Careful examination of allele frequencies revealed that no loci demonstrated clinal variation along elevational transects. For my purposes, a cline would be demonstrated by the following criteria: 1) the locus in consideration was polymorphic (most common allele less than 95%), and 2) the allele frequencies of that locus increased or decreased in all three transects. If we change criteria and examine single transects, there were only 13 potential clines out of a possible 87 (Table 3). Allele frequencies in these possible clines either increased or decreased in their respective transects. If loci that showed an increase in one transect and a decrease in another transect were eliminated, only eight of the 87 indicated any possible clinal variation. Since the probability of showing clinal variation

Table 3. Allelic variation at 29 gene loci in nine populations of lodgepole pine in Alberta.

Locus and Allele	Relative Migration of Allele	POPULATION 1	2	3	4	5	6	7	8	9
AAT-0										
1	100	0.990	1.000	1.000	1.000	1.000	1.000	1.000	0.990	1.000
2	105	0.010	0.0	0.0	0.0	0.0	0.0	0.0	0.010	0.0
E(h)		0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.02	0.0
O(h)		0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.02	0.0
AAT-1										
1	100	0.939	0.898	0.939	0.949	0.929	0.969	0.908	0.959	0.939
2	120	0.061	0.092	0.061	0.051	0.071	0.031	0.092	0.041	0.061
3	107	0.0	0.010	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E(h)		0.116	0.187	0.116	0.098	0.134	0.060	0.169	0.079	0.116
O(h)		0.122	0.163	0.082	0.102	0.143	0.061	0.184	0.082	0.122
		*	*	*	*	*		*	*	*
AAT-2										
1	100	0.969	0.898	0.990	0.908	0.878	0.908	0.949	0.827	0.918
2	40	0.020	0.041	0.010	0.082	0.092	0.051	0.051	0.092	0.071
3	89	0.0	0.010	0.0	0.0	0.031	0.041	0.0	0.061	0.010
4	113	0.010	0.051	0.0	0.010	0.0	0.0	0.0	0.020	0.0
E(h)		0.060	0.191	0.02	0.170	0.223	0.173	0.098	0.307	0.153
O(h)		0.061	0.204	0.02	0.184	0.204	0.184	0.102	0.347	0.143
			*		*	*	*	*	*	*
ACO-1										
1	100	0.490	0.663	0.714	0.663	0.694	0.704	0.541	0.439	0.510
2	89	0.143	0.031	0.061	0.010	0.010	0.020	0.122	0.041	0.082
3	84	0.194	0.194	0.204	0.204	0.255	0.245	0.245	0.439	0.327
4	75	0.173	0.112	0.020	0.122	0.041	0.031	0.092	0.082	0.082
E(h)		0.679	0.514	0.449	0.509	0.456	0.448	0.631	0.613	0.626
O(h)		0.429	0.469	0.429	0.490	0.306	0.429	0.510	0.408	0.408
			*	*	*	*	*	*	*	*
ADH-1										
1	100	0.663	0.520	0.449	0.449	0.398	0.449	0.316	0.388	0.408
2	120	0.316	0.459	0.551	0.510	0.541	0.520	0.663	0.602	0.561
3	110	0.020	0.020	0.0	0.041	0.061	0.031	0.0	0.010	0.031
4	125	0.0	0.0	0.0	0.0	0.0	0.0	0.020	0.0	0.0
E(h)		0.464	0.523	0.500	0.542	0.551	0.532	0.464	0.492	0.523

O(h)		0.306 *	0.571 *	0.571 *	0.490 *	0.673 *	0.592 *	0.469 *	0.510 *	0.490 *
AKK-1										
1	100	0.918	0.980	0.980	1.000	0.990	0.949	0.949	0.990	1.000
2	91	0.082	0.020	0.020	0.0	0.010	0.051	0.051	0.010	0.0
E(h)		0.151	0.040	0.040	0.0	0.02	0.098	0.098	0.02	0.0
O(h)		0.163 *	0.041	0.041	0.0	0.02	0.061 *	0.02 *	0.02	0.0
ALD-1										
1	100	0.673	0.806	0.816	0.908	0.867	0.633	0.714	0.724	0.827
2	125	0.316	0.194	0.184	0.092	0.133	0.367	0.286	0.276	0.173
3	93	0.010	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E(h)		0.451	0.316	0.303	0.169	0.232	0.470	0.412	0.403	0.290
O(h)		0.204 *	0.265 *	0.204 *	0.102 *	0.265 *	0.327 *	0.163 *	0.184 *	0.143 *
APH-1										
1	100	0.673	0.622	0.622	0.500	0.724	0.704	0.602	0.582	0.735
2	120	0.276	0.245	0.378	0.500	0.276	0.296	0.398	0.418	0.265
3	145	0.051	0.133	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E(h)		0.473	0.541	0.475	0.505	0.403	0.421	0.484	0.492	0.394
O(h)		0.449	0.449 *	.0469	0.510	0.265 *	0.224 *	0.429 *	0.469 *	0.367 *
APH-2										
1	100	0.949	0.990	1.000	1.000	1.000	1.000	1.000	0.990	1.000
2	74	0.051	0.010	0.0	0.0	0.0	0.0	0.0	0.010	0.0
E(h)		0.098	0.02	0.0	0.0	0.0	0.0	0.0	0.02	0.0
O(h)		0.02 *	0.02	0.0	0.0	0.0	0.0	0.0	0.02	0.0
APH-3										
1	100	0.480	0.561	0.520	0.582	0.724	0.541	0.500	0.551	0.673
2	105	0.061	0.133	0.122	0.092	0.082	0.082	0.122	0.316	0.173
Null		0.459	0.306	0.357	0.327	0.194	0.378	0.378	0.133	0.153
E(h)		0.561	0.580	0.593	0.552	0.435	0.564	0.599	0.585	0.498
O(h)		0.735 *	0.653 *	0.469 *	0.204 *	0.184 *	0.306 *	0.469 *	0.285 *	0.449 *
CEE-1										
1	100	0.735	0.653	0.949	0.969	0.969	0.969	0.898	0.847	0.867
2	96	0.265	0.347	0.051	0.031	0.031	0.031	0.102	0.153	0.133
E(h)		0.394	0.458	0.098	0.06	0.06	0.06	0.185	0.262	0.232
O(h)		0.163 *	0.082 *	0.102 *	0.061	0.061	0.02 *	0.163 *	0.102 *	0.143 *
DIA-1										
1	100	0.816	0.939	0.929	0.990	0.959	0.969	0.980	0.990	1.000

2	80	0.153	0.010	0.051	0.010	0.020	0.0	0.020	0.010	0.0
3	89	0.0	0.041	0.0	0.0	0.0	0.020	0.0	0.0	0.0
4	120	0.031	0.010	0.020	0.0	0.020	0.010	0.0	0.0	0.0
E(h)		0.312	0.118	0.136	0.02	0.08	0.06	0.04	0.02	0.0
O(h)		0.041	0.02	0.122	0.02	0.061	0.02	0.041	0.02	0.0
		*	*	*		*	*			
FLE-1										
1	100	0.867	0.735	0.990	0.929	0.908	0.898	0.827	0.959	0.898
2	96	0.051	0.265	0.010	0.071	0.092	0.102	0.163	0.041	0.092
3	109	0.082	0.0	0.0	0.0	0.0	0.0	0.010	0.0	0.010
E(h)		0.241	0.394	0.02	0.134	0.169	0.185	0.293	0.079	0.187
O(h)		0.102	0.122	0.02	0.143	0.143	0.122	0.184	0.082	0.122
		*	*		*	*	*	*	*	*
GDH-1										
1	100	1.000	0.990	1.000	0.990	0.990	0.959	0.990	0.990	1.000
2	84	0.0	0.010	0.0	0.010	0.010	0.041	0.010	0.010	0.0
E(h)		0.0	0.02	0.0	0.02	0.02	0.079	0.02	0.02	0.0
O(h)		0.0	0.02	0.0	0.02	0.02	0.041	0.02	0.02	0.0
							*			
G6P-1										
1	100	1.000	0.949	0.888	0.990	0.929	0.908	0.796	0.837	0.857
2	94	0.0	0.051	0.112	0.010	0.071	0.092	0.204	0.163	0.143
E(h)		0.0	0.098	0.201	0.02	0.134	0.169	0.328	0.276	0.247
O(h)		0.0	0.102	0.224	0.02	0.143	0.184	0.367	0.204	0.286
			*	*		*	*	*	*	*
HAA-1										
1	100	0.837	0.592	0.898	0.908	0.806	0.837	0.520	0.663	0.765
2	87	0.163	0.408	0.102	0.092	0.194	0.163	0.480	0.337	0.235
E(h)		0.276	0.488	0.185	0.169	0.316	0.276	0.504	0.451	0.363
O(h)		0.286	0.204	0.204	0.143	0.184	0.204	0.265	0.224	0.184
		*	*	*	*	*	*	*	*	*
HKK-1										
1	100	0.633	0.980	1.000	1.000	0.980	0.990	1.000	1.000	0.959
2	83	0.367	0.020	0.0	0.0	0.020	0.010	0.0	0.0	0.041
E(h)		0.470	0.04	0.0	0.0	0.04	0.02	0.0	0.0	0.079
O(h)		0.0	0.0	0.0	0.0	0.0	0.02	0.0	0.0	0.0
		*	*			*				*
IDH-1										
1	100	0.990	0.969	0.990	0.969	1.000	0.990	1.000	0.990	1.000
2	126	0.010	0.031	0.010	0.031	0.0	0.010	0.0	0.0	0.0
3	153	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	67	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.010	0.0
E(h)		0.02	0.06	0.02	0.06	0.0	0.02	0.0	0.02	0.0
O(h)		0.02	0.061	0.02	0.061	0.0	0.02	0.0	0.02	0.0

LAP-1										
1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-1										
1	100	0.990	0.980	0.990	0.990	0.990	0.990	0.980	0.990	1.000
2	71	0.010	0.020	0.010	0.010	0.010	0.010	0.020	0.010	0.0
E(h)		0.02	0.04	0.02	0.02	0.02	0.02	0.04	0.02	0.0
O(h)		0.02	0.041	0.02	0.02	0.02	0.02	0.041	0.02	0.0
MDH-2										
1	100	0.990	0.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2		0.010	0.010	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E(h)		0.02	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0
O(h)		0.02	0.02	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MDH-3										
1	100	0.622	0.653	0.673	0.643	0.622	0.714	0.551	0.694	0.735
2	58	0.224	0.235	0.133	0.153	0.265	0.173	0.173	0.143	0.194
3	85	0.102	0.0	0.0	0.204	0.112	0.112	0.010	0.163	0.0
4	44	0.051	0.020	0.020	0.0	0.0	0.0	0.265	0.0	0.010
Null		0.0	0.092	0.173	0.0	0.0	0.0	0.0	0.0	0.061
E(h)		0.555	0.515	0.503	0.527	0.535	0.452	0.602	0.476	0.423
O(h)		0.367 *	0.429 *	0.510	0.449 *	0.408 *	0.449 *	0.469 *	0.388 *	0.347 *
MDH-4										
1	100	0.990	0.990	1.000	0.969	0.980	0.980	0.980	0.980	0.990
2	175	0.010	0.010	0.0	0.020	0.020	0.020	0.020	0.020	0.010
3	105	0.0	0.0	0.0	0.010	0.0	0.0	0.0	0.0	0.0
E(h)		0.02	0.02	0.0	0.06	0.04	0.04	0.04	0.04	0.02
O(h)		0.02	0.02	0.0	0.061	0.041	0.041	0.041	0.041	0.02
PGM-1										
1	100	0.969	0.959	0.990	0.990	0.980	0.990	0.939	0.990	0.990
2	105	0.031	0.041	0.010	0.010	0.020	0.010	0.061	0.010	0.010
E(h)		0.06	0.079	0.02	0.02	0.04	0.02	0.116	0.02	0.02
O(h)		0.061	0.082 *	0.02	0.02	0.041	0.02	0.122 *	0.02	0.02
PGI-1										
1	100	1.000	1.000	1.000	1.000	0.990	1.000	1.000	1.000	1.000
2	127	0.0	0.0	0.0	0.0	0.010	0.0	0.0	0.0	0.0
E(h)		0.0	0.0	0.0	0.0	0.02	0.0	0.0	0.0	0.0
O(h)		0.0	0.0	0.0	0.0	0.02	0.0	0.0	0.0	0.0

PGI-2										
1	100	0.969	0.990	0.969	0.980	0.949	0.959	0.959	0.929	0.980
2	110	0.031	0.0	0.031	0.020	0.051	0.041	0.041	0.071	0.020
3	150	0.0	0.010	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E(h)		0.06	0.02	0.06	0.04	0.098	0.079	0.079	0.134	0.04
O(h)		0.061	0.02	0.061	0.041	0.102	0.082	0.041	0.143	0.041
						*	*	*	*	
6PG-1										
1	100	0.990	0.980	0.980	0.990	0.990	0.990	0.959	0.969	0.969
2	93	0.010	0.020	0.020	0.010	0.010	0.010	0.041	0.031	0.031
E(h)		0.06	0.04	0.04	0.02	0.02	0.02	0.079	0.06	0.06
O(h)		0.061	0.041	0.041	0.02	0.02	0.02	0.082	0.061	0.061
								*		
6PG-2										
1	100	0.980	0.990	0.990	0.980	1.000	0.990	0.959	0.990	1.000
2	84	0.010	0.010	0.010	0.020	0.0	0.010	0.041	0.010	0.0
3	114	0.010	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E(h)		0.04	0.02	0.02	0.04	0.0	0.02	0.079	0.02	0.0
O(h)		0.041	0.02	0.02	0.041	0.0	0.02	0.082	0.02	0.0
								*		
SDH-1										
1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.990	1.000
2	70	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.010	0.0
E(h)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.02	0.0
O(h)		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.02	0.0

E(h) = expected heterozygosity
O(h)* = observed heterozygosity
* = significant deviation from Hardy-Weinberg equilibrium

Table 4. Summary of heterogeneity chi-square analysis for 28 gene loci of lodgepole pine in Alberta.

Locus	Over all nine Populations	Transect Means	Elevation Means
AAT-0	nt	nt	nt
AAT-1	ns	ns	ns
AAT-2	*	*	*
ACO	*	*	*
ADH	*	*	ns
AK	*	ns	ns
ALD	*	ns	ns
APH-1	*	*	*
APH-2	nt	*	ns
APH-3	*	*	*
CE	*	*	*
DIA	*	*	*
FLE	*	*	*
GDH	ns	ns	ns
G6P	*	*	*
HA	*	*	*
HK	*	*	*
IDH	ns	ns	ns
MDH-1	ns	ns	ns
MDH-2	nt	nt	nt
MDH-3	*	*	*
MDH-4	ns	ns	ns
PGM	ns	ns	ns
PGI-1	nt	nt	nt
PGI-2	ns	ns	ns
6PG-1	ns	ns	ns
6PG-2	ns	ns	ns
SDH	nt	nt	nt
Total Chi-square	1428.191*	482.965*	359.375*

* = significant
ns = not significant
nt = insufficient variation for test to be made

Table 5. Summary of heterogeneity chi-square analysis for 28 gene loci of lodgepole pine in Alberta.

Locus	Robb Mid vs. Robb Mid center plot	Nordegg Low vs. Nordegg Low center plot	Cochrane Low vs. Cochrane Low center plot
AAT-0	nt	nt	nt
AAT-1	ns	ns	ns
AAT-2	ns	ns	*
ACO	*	*	ns
ADH	ns	ns	*
AK	nt	nt	ns
ALD	*	*	*
APH-1	ns	*	ns
APH-2	nt	ns	*
APH-3	ns	*	ns
CE	*	*	ns
DIA	ns	nt	nt
FLE	*	ns	*
GDH	nt	nt	nt
G6P	ns	*	ns
HA	*	*	*
HK	nt	nt	nt
IDH	nt	*	nt
MDH-1	nt	nt	nt
MDH-2	nt	nt	nt
MDH-3	*	*	*
MDH-4	nt	nt	ns
PGM	nt	nt	ns
PGI-1	nt	nt	nt
PGI-2	*	nt	ns
6PG-1	nt	nt	ns
6PG-2	nt	nt	ns
SDH	nt	nt	nt
Total Chi-square	111.908*	221.311*	112.591*

* = significant
ns = not significant
nt = insufficient variation for test to be made

Table 6. Common allele frequencies of selected loci of lodgepole pine averaged over three latitudinal transects in Alberta.

		Transect		
Locus	Allele	Robb	Nordegg	Cochrane
AAT-2	1	.953	.898	.898
ADH	1	.545	.432	.371
PGI-2	1	.976	.963	.956

Table 7. Common allele frequencies of selected loci of lodgepole pine averaged over three elevations in Alberta.

		Elevation		
Locus	Allele	Low	Mid	High
ACO	1	.565	.599	.643
APH-1	1	.592	.643	.687
DIA	1	.929	.964	.966
G6P	1	.966	.905	.833
MDH-3	1	.605	.656	.708

over the three plots of one transect is 0.33, the proportion exhibiting clinal variation is less than expected by chance.

The percentage of polymorphic loci ranged from 58.6 to 86.2 (Table 8). A locus was considered polymorphic if more than one allele was detected in any population. Along transects, the mean number of polymorphic loci decreased from the Robb transect to the Cochrane transect. In addition, the high elevation populations were the least polymorphic when the average was taken by elevation.

Mean expected heterozygosity ranged from 0.130 to 0.193, but did not show significant heterogeneity among the nine populations sampled (Table 8). Approximately 58 percent of the loci tested showed a significant deviation from the expected Hardy-Weinberg equilibrium proportions (Table 3). Thirty-five percent of these departures were due to a heterozygote excess, with the remainder due to a deficiency of heterozygotes. In all the populations, mean expected heterozygosity always exceeded the observed number of heterozygotes, as indicated by the positive mean F(is) estimate (Table 9).

Only 4.7 percent of the detected genic variation was due to inter-population differences, with the remaining 95.3 percent residing within populations. The F(st) values ranged from 0.007 to 0.262 over the 28 loci examined (Table 9).

Mean genetic distance among the nine populations was 0.008, with a range from 0.002 to 0.020 (Table 10). There was no significant correlation between genetic distance and

Table 8. Genetic variability measures of nine lodgepole pine populations in Alberta.

Population	Mean No. of Alleles Per Locus	Percentage of Loci Polymorphic	Mean Heterozygosity	
			Direct Count	HDYWBG** Expected
Robb Low	2.2 (0.2)*	82.8	.129 (0.032)	.193 (0.039)
Robb Mid	2.3 (0.2)	86.2	.142 (0.035)	.184 (0.039)
Robb High	1.9 (0.2)	69.0	.126 (0.034)	.132 (0.035)
Nordegg Low	2.0 (0.1)	72.4	.110 (0.030)	.130 (0.036)
Nordegg Mid	2.0 (0.1)	75.9	.115 (0.029)	.140 (0.033)
Nordegg High	2.0 (0.1)	79.3	.120 (0.030)	.148 (0.035)
Cochrane Low	2.0 (0.2)	72.4	.147 (0.032)	.185 (0.040)
Cochrane Mid	2.1 (0.1)	86.2	.128 (0.029)	.171 (0.039)
Cochrane High	1.8 (0.2)	58.6	.115 (0.028)	.147 (0.036)

* standard errors in parentheses

** Hardy-Weinberg, unbiased estimate Nei (1978)

Table 9. Estimates of F-statistics at 28 loci of nine lodgepole pine populations in Alberta.

LOCUS	F(IS)	F(IT)	F(ST)
AAT-0	-0.010	-0.002	0.008
AAT-1	0.003	0.010	0.007
AAT-2	-0.049	-0.024	0.023
ACO-1	0.204	0.233	0.036
ADH-1	-0.028	0.006	0.033
AKK-1	0.208	0.229	0.026
ALD-1	0.384	0.410	0.043
APH-1	0.124	0.149	0.029
APH-2	0.554	0.568	0.032
APH-3	0.240	0.270	0.039
CEE-1	0.498	0.550	0.103
DIA-1	0.555	0.580	0.057
FLE-1	0.382	0.416	0.055
GDH-1	0.203	0.214	0.014
G6P-1	-0.049	0.003	0.049
HAA-1	0.367	0.424	0.091
HKK-1	0.968	0.977	0.262
IDH-1	-0.023	-0.010	0.012
MDH-1	-0.014	-0.011	0.003
MDH-2	-0.010	-0.002	0.008
MDH-3	0.160	0.192	0.038
MDH-4	-0.019	-0.015	0.004
PGM-1	-0.037	-0.023	0.013
PGI-1	-0.010	-0.001	0.009
PGI-2	0.022	0.032	0.010
6PG-1	-0.027	-0.021	0.006
6PG-2	-0.023	-0.013	0.011
SDH-1	-0.010	-0.001	0.009
MEAN	0.199	0.237	0.047

Table 10. Genetic distance (below the diagonal; from Nei 1978) and geographic distance (km; above the diagonal) for pairwise comparisons among nine lodgepole pine populations in Alberta.

	POPULATION								
	1	2	3	4	5	6	7	8	9
1	-	30	35	105	110	115	230	205	195
2	.013	-	15	100	95	110	225	200	210
3	.013	.010	-	115	110	120	235	215	220
4	.015	.012	.002	-	15	20	120	95	90
5	.015	.008	.003	.003	-	15	125	95	100
6	.011	.009	.002	.004	.003	-	115	90	95
7	.020	.008	.009	.012	.009	.008	-	25	30
8	.019	.010	.008	.008	.006	.007	.006	-	20
9	.014	.006	.004	.006	.001	.004	.007	.002	-

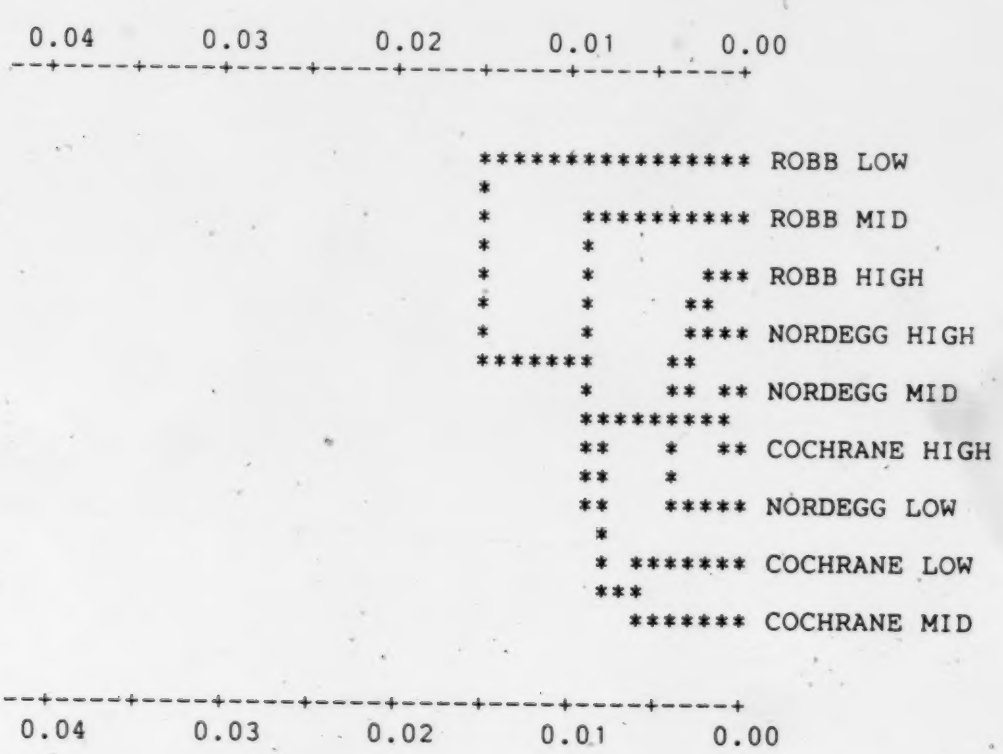
The correlation between genetic distance and geographic distance = 0 .02

geographic distance. As revealed by phenetic clustering, the Robb Low population (number one) was most different from all other populations (Figure 3).

A discriminant analysis using the populations to define groups was performed using the most common allele of every variable gene locus. After completion of a principal components analysis, five factor groups were chosen to represent the factor loadings of the genetic data. The univariate F-ratio showed that one factor group was significant.

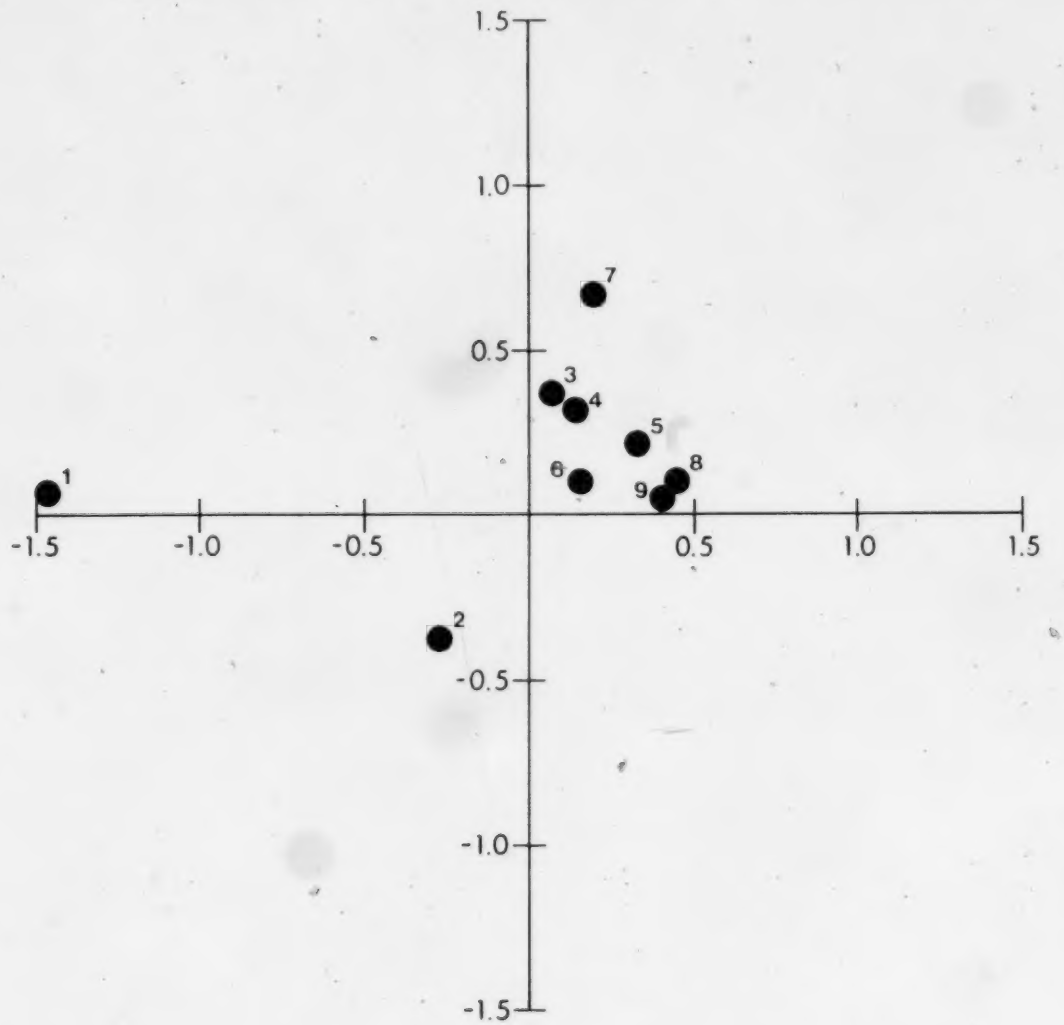
A total of five canonical discriminant functions were derived from the data, of which three were significant. When plotted, these canonical discriminant function values suggested that the centroid for population 1 may be significantly different from the other eight populations. (Figure 4).

Figure 3. UPGMA'-derived dendrogram of nine lodgepole pine populations in Alberta based on Nei's (1978) unbiased genetic distance measure.



'Unweighted pair group method with arithmetic averaging

Figure 4. Population centroids on the first two discriminant axes.



IV. DISCUSSION

Levels of heterozygosity observed in the nine lodgepole pine populations are within the range of values previously reported for lodgepole pine (Dancik and Yeh 1983, Wheeler and Guries 1982, and Yeh and Layton 1979) and other conifers (Guries and Ledig 1981, Hamrick *et al.* 1981, O'Malley *et al.* 1979, Yeh and El-Kassaby 1980, and Yeh and O'Malley 1980). The mean percentage of loci polymorphic over all populations was 75.87, which is high compared to the values of 58.67% (Yeh and Layton 1979), 69.0% (Wheeler and Guries 1982), and 51.4% (Dancik and Yeh 1983) reported in previous studies of lodgepole pine. However, this may be misleading, since many of the polymorphic loci only had two alleles and the most common allele predominated. Also, this study assumed a locus was polymorphic if more than one allele was detected in any population, while the other studies used 95% and 99% criteria. The mean number of alleles per locus over all populations was 2.03, which is in accordance with other reported values. In other lodgepole pine studies, the mean number of alleles per locus ranged from 1.86 (Wheeler and Guries 1982) to 2.74 (Conkle 1981).

The mean observed heterozygosity for all nine populations studied was 0.126. This compares to values of 0.138 for pitch pine *Pinus rigida* Mill. (Guries and Ledig 1981), 0.123 for ponderosa pine *Pinus ponderosa* Laws. (O'Malley *et al.* 1979) 0.155 for Douglas-fir *Pseudotsuga*

menziesii ssp. *menziesii* (Mirb.) Franco (Yeh and O'Malley 1980), 0.150 for Sitka spruce *Picea sitchensis* (Bong.) Carr. (Yeh and El-Kassaby 1980), and 0.161 (Yeh and Layton 1979), 0.116 (Wheeler and Guries 1982), 0.184 (Dancik and Yeh 1983) and 0.185 (Conkle 1981) for lodgepole pine in other studies.

In all nine populations, the expected heterozygosity exceeded observed heterozygosity. This may have been due to inbreeding due to related matings. It has been suggested that a majority of seeds of coniferous trees tend to fall and grow near their maternal parents (Libby *et al.* 1969). The population can thus become subdivided into a number of family clusters. Pollination within these clusters theoretically would decrease the number of heterozygotes formed. A small level of inbreeding, about five percent each generation, could contribute to a heterozygote deficiency in a population. Another possible explanation for the deficiency of heterozygotes is the Wahlund effect (Wahlund 1928), in which pooling of individuals from several breeding groups, each with different allele frequencies, leads to apparent deficiency of heterozygotes.

Brown (1979) has listed other factors, such as partial self-pollination, null alleles, negative heterosis, and dominant alleles at modifier loci, which could lead to deficiency of heterozygotes. Perry (1985) found very high outcrossing rates in three of the nine populations examined in this study, indicating that partial self-pollination is likely not a cause of heterozygote deficiency in this case.

Family structure is a more likely explanation. Evidence of family structure within the nine lodgepole pine populations studied was indicated by inbreeding within stands, probably due to related matings and by the fact that there was significant heterogeneity of allele frequencies between the 10 m x 10 m center plot and the 49 tree plot at three of the populations. Microgeographic differentiation and family structure within populations have been reported in lodgepole pine (Knowles 1980) and other conifers (Mitton *et al.* 1977, Linhart *et al.* 1981a, 1981b).

Little genetic differentiation was observed among populations of lodgepole pine, with 4.7% of the genic diversity among these loci residing among populations. Similar F(st) results also have been reported in other conifer species (Table 11).

The low degree of allozyme differentiation among populations of coniferous species may be due to a number of factors, including their potential for long distance pollen and seed dispersal (Stern and Roche 1974), high outcrossing rates (Mitton *et al.* 1977), general intolerance of selfing (Wright 1976), and formation of extensive populations. A high proportion of variation within populations may be an adaptive response to spatial and temporal environmental heterogeneity encountered by individual populations (Campbell 1979).

Despite the low proportion of variation among populations, significant heterogeneity of allele frequencies

Table 11. F(st) statistics from comparable studies of coniferous trees.

Species	F(st)	Study
Pinus contorta ssp *latifolia*	0.041	Yeh and Layton (1979)
"	0.032	Wheeler and Guries (1982)
"	0.043	Dancik and Yeh (1983)
"	0.047	This study
Pinus longaeva Bailey.	0.038	Hiebert and Hamrick (1983)
Picea sitchensis	0.079	Yeh and El'Kassaby (1980)
Pinus rigida	0.024	Guries and Ledig (1982)
Pseudotsuga menziesii	0.026	Yeh and O'Malley (1980)
Pinus banksiana Lamb.	0.024	Dancik and Yeh (1983)

was observed among populations, among transects, and among elevations. Greater heterogeneity among transects than elevations was found; this may be due to the greater geographic distances involved, since the transects are farther apart geographically than are the three elevational plots within one transect. However, geographic distance was not significantly correlated with genetic distance in this study.

One possible cause of the observed heterogeneity of gene frequencies among populations is differential selection pressures on different populations, reflecting heterogeneity in the environments from which the trees were sampled. Another possible cause is genetic drift, leading to random differentiation of populations. The continuous distribution of lodgepole pine in the foothills of Alberta makes this unlikely, since a lack of barriers to gene flow would be expected to counteract differentiation due to genetic drift.

This study has shown that there was evidence of family structure within these populations of lodgepole pine. This was indicated by high levels of inbreeding within stands, probably due to related matings, and significant allele heterogeneity found among the center plot and all trees studied in three of the nine populations. Family structure may, then, occur in continuous stands, contrary to the report of Koski (1974).

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VI. APPENDIX I.

A. STAND DESCRIPTIONS

Table I. Stand summary data for nine lodgepole pine populations in Alberta.

Population[2]	Ave. Age	Ave. Height (m)	Ave. diameter (cm)	Basal area (m^2/ha)
Robb Low	80.41 (4.81)[3]	22.43 (4.73)	22.07 (2.37)	31.49 (3.31)
Robb Mid	84.20 (9.17)	19.94 (1.73)	23.00 (3.93)	29.90 (3.39)
Robb High	75.00 (3.49)	13.59 (1.42)	18.15 (2.91)	35.39 (4.66)
Nordegg Low	80.31 (3.70)	16.36 (1.61)	17.42 (2.77)	25.77 (4.20)
Nordegg Mid	84.31 (11.85)	13.84 (2.59)	14.16 (1.38)	39.94 (3.42)
Nordegg High	108.50 (10.42)	11.87 (0.73)	15.41 (1.33)	58.20 (4.29)
Cochrane Low	89.27 (3.47)	15.33 (1.11)	15.12 (2.15)	28.35 (4.75)
Cochrane Mid	108.94 (14.93)	17.66 (4.20)	18.33 (1.74)	22.48 (3.42)
Cochrane High	109.76 (10.47)	9.46 (1.02)	15.54 (2.79)	48.49 (7.62)

[2]The nine populations sampled were continuous stands of lodgepole pine. A variety of slopes and aspects was chosen as well. A number of trees sampled were infested with western gall rust (*Endocronartium harknessii* Moore (Hiratsuka) or the blue stain fungus (*Ceratocystis pini* Munch). Infested trees showed a decrease in vigor and were perhaps near death. The Robb transect had more cases of diseased and forked trees than the other two transects.
[3]standard deviations

VII. APPENDIX II.

A. BUFFERS USED IN STAINING SOLUTIONS

(1) **0.2 M Acetate, pH 5.0**

sodium acetate.....27.22 g

distilled water.....800 ml

adjust pH with concentrated glacial acetic acid and distilled water to 1L

(2) **0.05 M Citrate - 0.05 M Phosphate, pH 4.0**

sodium phosphate (dibasic).....11.41g

distilled water.....1L

adjust pH with 1M Citric acid

(3) **0.2 M Tris - HCL pH 8.0**

Trizma base.....24.22 g

distilled water.....1L

adjust pH with concentrated hydrochloric acid

(4) **0.2 M Tris maleate**

0.2 M Tris.....50 ml approx.

0.2 M Maleic anhydride.....50 ml approx.

mix together and adjust pH to either 5.3 for LAP or 6.4 for CE with appropriate solution

(5) 0.1 M Tris - HCL pH 7.1

Trizma base.....12.11 g

distilled water.....1L

adjust pH with concentrated hydrochloric acid

B. ENZYME STAINING RECIPES

Enzyme staining recipes were used directly from or modified slightly from Lin *et al.* (1969), Mitton *et al.* (1979), Shaw and Prasad (1970), Siciliano and Shaw (1976), Yeh and Layton (1979), and Yeh and O'Malley (1980).

(1) ASPARTATE AMINOTRANSFERASE (AAT)

Siciliano and Shaw (1976) E.C. 2.0.1.1.

Stain buffer: 3 (50 ml)

Fast Blue BB Salt(100mg/ml).....2 ml

AAT Substrate Solution: (50 ml)

α-ketoglutaric acid.....146 mg

L-aspartic acid.....532 mg

polyvinylpyrrolidine.....2 mg

ethylenediaminetetra-acetic acid (EDTA)0.2 g

NA_2HPO_4.....5.68 g

distilled water to 200 ml

Add Fast Blue BB Salt just before staining. Incubate gels at 37° C in dark.

(2) ACONITASE (ACO)

Yeh and O'Malley (1980) E.C. 4.2.1.3.

Stain buffer:3 (80 ml)

NADP (10mg/ml).....4 ml

$MgCl_2$.....2 ml

NBT (10mg/ml).....2 ml

PMS (10mg/ml).....2 ml

MTT (10mg/ml).....2 ml

IDH.....80 u

Cis-aconitic acid (5mg/100ml) pH 7.0 w NAOH...10 ml

Incubate at 37° C in dark.

(3) **ALCOHOL DEHYDROGENASE (ADH)**

Shaw and Prasad (1970) E.C. 1.1.1.1.

Stain buffer:3 (74 ml)

NAD (10mg/ml).....2 ml

MTT.....2 ml

PMS.....2 ml

95% ethanol.....1-2 ml

Incubate at 37° C in dark.

(4) **ADENYLATE KINASE (AK)**

Yeh and Layton (1979) E.C. 2.7.4.3.

Stain buffer:3 (90 ml)

NADP.....2 ml

$MgCl_2$.....2 ml

PMS.....2 ml

Glucose.....100 mg

ADP.....100 mg

Hexokinase.....500 u

Glucose-6-phosphate dehydrogenase (50u/ml)....2 ml

Incubate at 37° C in dark.

(5) **ALDOLASE (ALD)**

Yeh and O'Malley (1980) E.C. 4.1.2.13.

Stain buffer:3 (94 ml)

NAD.....2 ml

MTT.....2 ml

PMS.....2 ml

Fructose-1,6-diphosphate.....500 mg

Arsenic acid.....150 mg

Glyceraldehyde-3-phosphate dehydrogenase.....600 u

Incubate at 37° C in dark.

(6) **ACID PHOSPHATASE (APH)**

Yeh and Layton (1979) E.C. 3.1.3.2.

Stain buffer:1 (90 ml)

$MgCl_2$.....2 ml

Fast Garnet GBC Salt.....150 mg

Na-α-naphthyl acid phosphate.....300 mg

Incubate at 37° C in dark.

(7) **COLORIMETRIC ESTERASE (CE)**

Shaw and Prasad (1970) E.C. 3.1.1.1.

Stain buffer:4 (pH 6.4 100 ml)

α-naphthyl acetate.....40 mg

Fast Blue BB Salt.....2 ml

Incubate in dark at room temperature.

(8) **DIAPHORASE (DIA)**

Yeh and O'Malley (1980) E.C. 1.6.4.3.

Stain buffer:3 (50 ml)

MTT.....2 ml

NADH.....50 mg

2,6-dichlorophenol indophenol.....2 mg

Agar overlay:

Tris.....50 ml

Agar.....1 g

Melt agar, cool and add substrate solution. Incubate at 37° C in dark.

(9) **FLOURESCENT ESTERASE (FLE)**

Mitton *et al*. (1979) E.C. unclassified

Stain buffer:1 (24 ml)

Acetone.....5 ml

α-methylumbelliferyl acetate.....10 mg

Dissolve the α-methylumbelliferyl acetate in the acetone. Add to the stain buffer and mix well. Paint staining solution on the gel and incubate at room temperature for approximately 10 minutes. View under long wave (375 nm) ultra-violet light (U.V.) in the dark. Zones of flourescence indicate areas of enzyme activity.

(10) **GLUTAMATE DEHYDROGENASE (GDH)**

Yeh and O'Malley (1980) E.C. 1.4.1.3.

Stain buffer:3 (90 ml)

NAD.....6 ml

PMS.....2 ml

NBT.....2 ml

MTT.....2 ml

L-glutamic acid.....800 mg

Incubate at 37° C in dark.

(11) **GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6P)**

Yeh and Layton (1979) E.C. 1.1.1.49.

Stain buffer:3 (90 ml)

NADP.....4 ml

$MgCl_2$.....2 ml

MTT.....2 ml

PMS.....2 ml

Glucose-6-phosphate.....200 mg

Incubate at 37° C in dark.

(12) **HEXOSEAMINIDASE (HA)**

Siciliano and Shaw (1976) E.C. 3.2.1.30.

Stain buffer:2 (20 ml)

4 methylumbelliferyl-N-acetyl-β-D-glucosaminide.. 40 mg

Dissolve the substrate in the stain buffer. Paint the gel and incubate for approximately 40 minutes at room temperature. Sprinkle with concentrated NH_4OH and view the gel under long wave U.V. light in the dark. Zone of flourescence indicates areas of enzyme activity.

(13) **HEXOKINASE (HK)**

Shaw and Prasad (1970) E.C. 2.7.1.1.

Stain buffer:5 (100 ml)

NADP.....2 ml

$MgCl_2$.....2 ml

PMS.....2 ml

MTT.....2 ml

Glucose.....1 g

ATP.....25 mg

G-6-PDH.....2 ml

Incubate at 37° C in dark.

(14) **ISOCITRATE DEHYROGENASE (IDH)**

Yeh and O'Malley (1980) E.C. 1.1.1.41.

Stain buffer:3 (92 ml)

NADP.....2 ml

$MgCl_2$.....2 ml

MTT.....2 ml

PMS.....2 ml

DL-Isocitric acid.....400 mg

Incubate at 37° C in dark.

(15) **LEUCINE AMINOPEPTIDASE (LAP)**

Shaw and Prasad (1970) E.C. 3.4.11.1.

Stain buffer:4 (pH 5.3 100 ml)

$MgCl_2$.....2 ml

Fast Black K Salt.....140 mg

L-leucine-β-naphthyl-amide HCl.....100 mg

Dissolve substrates in acetone and add to stain buffer.

Incubate at 37° C in dark.

(16) **MALATE DEHYDROGENASE (MDH)**

Yeh and O'Malley (1980) E.C. 1.1.1.37.

Stain buffer:3 (44 ml)

DL-Malic acid.....50 ml

NAD.....4 ml

NBT.....2 ml

PMS.....2 ml

MTT.....2 ml

Incubate at 37° C in dark.

(17) **PHOSPHOGLUCOMUTASE (PGM)**

Yeh and O'Malley (1980) E.C. 2.7.5.1.

Stain buffer:3 (92 ml)

NADP.....2 ml

$MgCl_2$.....2 ml

MTT.....2 ml

PMS.....2 ml

Glucose-6-phosphate.....600 mg

Glucose-1,6-diphosphate.....1 ml

G-6-PDH.....1 ml

Incubate at 37° C in dark.

(18) **PHOSPHOGLUCOSE ISOMERASE (PGI)**

Yeh and O'Malley (1980) E.C. 5.3.1.9.

Stain buffer:3 (92 ml)

NADP.....2 ml

$MgCl_2$.....2 ml

MTT.....2 ml

PMS.....2 ml

G-6-PDH.....1 ml

Fructose-6-phosphate.....50 mg

Incubate at 37° C in dark.

(19) **6-PHOSPHOGLUCONIC DEHYDROGENASE (6PG)**

Yeh and O'Malley (1980) E.C. 1.1.1.44.

Stain buffer:3 (20 ml)

NADP.....4 ml

$MgCl_2$.....2 ml

MTT.....4 ml

PMS.....6 ml

Phosphogluconic acid (NA_3 salt.....20 mg

Incubate at 37° C in dark.

(20) **SORBITOL DEHYDROGENASE (SDH)**

Lin *et al.* (1969) E.C. 1.1.1.14.

Stain buffer:3 (100 ml)

NAD.....2 ml

MTT.....2 ml

PMS.....2 ml

Sorbitol.....2 g

Incubate at 37° C in dark.

C. GEL AND ELECTRODE BUFFERS

A. Electrode buffer: 63 g trizma base, 33.04 g citric acid (anhydrous), and 4 l distilled water. Adjust pH to 7.0 with HCL or NAOH; Gel buffer: 1:15 dilution of electrode buffer. Power: 200 v.

B. Electrode buffer: 74.2 g trizma base, 10.07 g LIOH, and 4 l distilled water. pH 8.1; Gel buffer: 76.2 g trizma base, 19.2 g citric acid (anhydrous), 200 ml B electrode buffer. pH 8.5. Power: 300 v.

C. Electrode buffer: 60.54 g trizma base and 4 l distilled water. Adjust pH to 7.0 with 1M citric acid (anhydrous); Gel buffer: 41.92 g L-Histidine HCL, 1.6 g EDTA, 4 l distilled water. Adjust pH to7.0 with 1M Tris. Power: 200 v.

D. Electrode buffer: 76.64 g trizma base, 10.08 g NAOH, and 4 l distilled water. Adjust pH to 8.1 with NAOH; Gel buffer: 9.68 g trizma base and 1 l distilled water. Adjust pH to 8.65 with 1M citric acid (anhydrous). Power: 300 v.